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Epstein-Barr Virus Infection in Richter's Transformation

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Chronic lymphocytic leukemia (CLL) may convert to a diffuse large cell lymphoma (Richter's syndrome) over time. In occasional cases of Richter's transformation, Epstein-Barr virus (EBV) has been identified in the lymphoma cells. To evaluate the association of EBV infection with Richter's syndrome, the biopsy specimens and clinical records of 25 patients who were seen at the Mayo Clinic between 1984–1996 were retrospectively evaluated for the presence of EBV by immunoperoxidase staining for expression of EBV latent membrane protein (LMP), as well as the expression of EBV RNA and DNA in the cells by in situ hybridization. Four of the 25 patients showed evidence of EBV in the diffuse large cell lymphoma cells—three patients with a B-cell phenotype were positive for LMP, EBV DNA, and RNA; and one patient with a T-cell phenotype had positive EBV RNA in the large cell lymphoma cells. The Richter's syndrome was treated with combination chemotherapy in 15 patients, three received radiotherapy, three were followed without further therapy after a splenectomy, two died before treatment could be started, and one patient had insufficient follow-up. One patient with evidence of EBV in large cell lymphoma cells was treated with acyclovir as initial therapy. The median survival of EBV-positive patients was three months compared with nine months for EBV-negative patients, but this difference was not statistically significant ($P = 0.385$). Evidence for EBV infection related to Richter's transformation was present in 16% of the patients in this study and may be associated with a poorer outcome. Primary therapy with acyclovir in one patient did not seem to be beneficial and other therapeutic modalities in patients with EBV-positive Richter's transformation need to be explored. Am J. Hematol. 60:99–104, 1999.

Key words: Epstein-Barr virus; Richter's transformation

INTRODUCTION

In 1928, Richter first described the occurrence of ''reticulum cell sarcoma'' in a patient with chronic lymphocytic leukemia (CLL) [1]. Since then, numerous investigators have sought to determine the clinical features of Richter's transformation, as well as the causal relationship between CLL and large cell lymphoma (LCL). In general, the development of a second higher-grade lymphoma, usually diffuse LCL or the immunoblastic variant, occurs in 1–10% of patients with CLL [2–5]. This is often characterized by sudden clinical deterioration as manifest by systemic symptoms, an increase in the serum lactate dehydrogenase (LDH), a rapid increase in lymphadenopathy, or extranodal involvement. Less commonly seen are a monoclonal gammopathy, lytic bone lesions, and multiple cytogenetic changes [2–6]. These findings are uncommon in CLL and survival rates are poor once this syndrome is diagnosed.

Two cases of Richter's transformation have been previously reported in which the large cells had a morphology similar to Reed-Sternberg cells and these cells were positive for EBV DNA as well as EBV latent membrane protein (LMP) [7]. A separate study of 10 cases of non-Hodgkin's lymphoma with Hodgkin and Reed-Sternberg–like cells found four cases, including one Richter's transformation, to be positive for EBV DNA as well as EBV LMP [8].

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In view of these findings, we retrospectively reviewed the clinical records of 25 patients with Richter's transformation and examined their stored biopsy specimens for the presence of EBV DNA, EBV RNA, and EBV LMP. The aim of this study was to evaluate the association of EBV infection with the clinical course and outcome of patients with Richter's transformation. A further aim was to compare the clinical course of EBV-positive patients with those who were EBV-negative.

Patients and Methods

Biopsy specimens and clinical records of patients with Richter's transformation seen between January 1984 and December 1996 were retrospectively reviewed. Patients were identified using the Mayo Clinic patient database. The patients were also cross-referenced using the lymphoma database and the surgical registry. All patients gave informed consent for the original biopsy and the study was approved by the institutional review board.

A total of 78 patients with Richter's transformation were seen at the Mayo Clinic during the study period. Twenty-five patients had histological material available for investigation and were included in the study. Patient characteristics are shown in Table I. Nineteen patients were male and six patients were female with a median age of 66 years (range: 39–85 years). All 25 patients had B-cell CLL prior to the diagnosis of Richter's transformation. Twenty-three patients developed B-cell diffuse LCL and two patients developed T-cell LCL. The median time from the diagnosis of CLL to the diagnosis of Richter's transformation was 59 months (range: 9–276 months). The sites of involvement by LCL are also shown in Table I.

In all cases, biopsy specimens were available for review. For light microscopy, all sections had been fixed in formalin and histologic sections were stained with hematoxylin-eosin. Immunohistologic studies were performed on paraffin-embedded tissues using a peroxidase labeled streptavidin-biotin method as described previously [9], with aminoethylcarbazole as the chromogen. The monoclonal antibodies used were Anti-CD3, Anti-CD20, Anti-kappa and Anti-lambda light chains, and Anti-LMP-1 (Dako, Carpinteria, CA).

EBV DNA was detected by in situ hybridization using a biotin labeled cDNA probe (Enzo Diagnostics, Formingdale, NY). Briefly, tissue sections on glass slides were digested with proteinase K, followed by acetylation with acetyl anhydride. The tissue sections were treated in prehybridization solution, followed by overnight incubation with the EBV probe. The slides were developed using antistreptavidin alkaline phosphatase (SAAP) followed by nitroblue tetrazolium and 5-bromo-4-chloro-3-indol phosphate (NBT/BCIP) with levamisole and counterstained with Nuclear Fast Red. Similarly, EBV RNA was detected with in situ hybridization using the DAKO ISH Detection System (Dako) as reported previously with positive and negative control slides [10].

TABLE I. Patient Characteristics*

	Number of patients
Number in the study	25
Gender	
Males	19
Females	6
Histology of Richter's Transformation	
B-cell diffuse large cell lymphoma	23
T-cell large cell lymphoma	2
EBV infection	
Positive	4
Negative	21
Sites of involvement	
Nodal involvement	17
Splenomegaly	10
Hepatomegaly	4
Pulmonary	4
Gastrointestinal	3
Central nervous system	3
Bone marrow	2
ENT involvement	2
Renal involvement	2
Subcutaneous	2

*EBV, Epstein-Barr virus; ENT, ears, nose, and throat.

Statistical analysis of distinct variables between EBV-positive and EBV-negative patients were compared by the χ^2 test. Survival duration was estimated by the Kaplan-Meier method [11], and statistical significance was determined by the log-rank test [12].

RESULTS

Four patients were found to be EBV positive (see Table II). Three of the four patients developed a B-cell lymphoma and one developed a T-cell lymphoma. The three B-cell lymphoma patients were positive for EBV DNA, EBV RNA, and LMP-1; and the T-cell lymphoma patient had positive EBV RNA in the LCL cells. The histological findings in one of the three patients with an EBV-associated B-cell lymphoma are shown in Figure 1. The histological findings in the fourth patient who developed an EBV-associated T-cell lymphoma is shown in figure 2. A fifth patient showed a few large cells with positive EBV RNA, but besides the monoclonal kappa B-cells (small and large cells), there were also intense CD3 positive small T-lymphocytes in the background. This picture was felt to be more compatible with EBV infection. The presence of an EBV infection could not be confirmed serologically as sera from this patient drawn at the time of the biopsy was not available.

Three of the four EBV-positive patients were male and one was female, aged 66, 80, 81, and 67 years, respectively. Three patients had received chlorambucil as

TABLE II. Characteristics of EBV-Positive Patients*

Gender	Age	Site of disease	Treatment	Outcome
Male	66 years	Lung	CHOP	Dead, other causes
Female	67 years	Axilla, abdomen	CHOP	Dead, from disease
Male	80 years	Neck, BM, spleen, liver	Acyclovir	Dead, from disease
Male	81 years	Tonsil, neck, inguinal	Radiotherapy	Alive

*EBV, Epstein-Barr virus; CHOP, Cyclophosphamide, doxorubicin, vincristine, and prednisone; BM, bone marrow.

therapy for their CLL and one patient had been followed without therapy. None of the patients had a history of treatment with fludarabine for their CLL. The site of involvement by the LCL was the lung in one patient; cervical adenopathy, hepatosplenomegaly, and bone marrow involvement in the second; tonsil, neck, and inguinal in the third; and axillary and abdominal lymphadenopathy in the fourth patient. When the diagnosis of Richter's transformation was made, two of the patients were treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), one patient received involved-field radiotherapy, and one patient received acyclovir as his initial therapy. Three patients have died, two from progressive disease and one from pneumonia after completing six cycles of therapy. One patient is alive, with persistent disease, 10 months after the diagnosis of Richter's transformation.

In the group of patients who were EBV-negative, 13 patients were treated with chemotherapy: Seven patients received CHOP; three patients received CVP (cyclophosphamide, vincristine, prednisone); one patient received DHAP (cisplatin, ara-C, dexamethasone); one patient received CPOB (cyclophosphamide, prednisone, vincristine, bleomycin); and one patient received nitrogen mustard. Two patients received radiotherapy to the site of lymphomatous involvement, and three patients whose disease was predominantly confined to the spleen had a splenectomy followed by observation. Two patients were untreated because of early death, and the follow-up data on one patient was insufficient.

The median survival for all patients was nine months (range: 1–49 months) and is shown in Figure 3. The survival of patients with confirmed EBV infection was three months and nine months for patients who were EBV-negative (see Figure 4). The difference in survival between these two groups, however, was not statistically significant ($P = 0.385$), probably because the number of patients in the EBV-positive group was very small.

DISCUSSION

CLL is a low-grade lymphoproliferative process that may convert to a higher grade neoplasm over time [1,4]. This higher-grade neoplasm is usually an LCL, but Hodgkin's-like Richter's transformation has been described [7]. The relationship between B-cell CLL and the subsequent higher-grade neoplasm is controversial. In more than half the cases, immunologic and molecular genetic studies have shown that the B-cell CLL and the higher-grade lymphoma are clonally related, with the latter representing histologic transformation [3,13–15]. However, in a subset of cases, the B-cell CLL and subsequent non-Hodgkin's lymphoma have different patterns of antigen expression or gene rearrangements, which suggests that the neoplasms may not be clonally related [3,13,15]. In occasional cases of Richter's syndrome, both clonally related and unrelated, EBV has been identified in the higher-grade neoplasm [8,13].

It has also previously been found that tissue from patients with CLL can contain pleomorphic cells resembling Reed-Sternberg cells and variants (RS-H) [3,16–21] seen in a background that was otherwise typical of CLL, without evidence of Richter's transformation. It has been shown that 40–90% of these cells, seen in patients with CLL but without clinical evidence of Richter's transformation, are positive for EBV [8,22,23]. Two of these reports have suggested that these cells may possibly transform to Hodgkin's disease and that this process may be mediated by EBV [22,23]. A few patients have been reported who had CLL and subsequently had a Richter's transformation to a Hodgkin's-like LCL [7,8,15,22]. In these reports, four cases of Richter's transformation with Hodgkin's-like cells were shown to be associated with EBV [7,8,15]. These findings have suggested to others that EBV may be involved in the transformation [8,22].

Despite the suggestion that EBV could be responsible for the Richter's transformation in a subset of patients, no studies have been done to determine the prevalence of EBV in patients with Richter's transformation. Furthermore, no studies have been done to compare the outcome of patients who were EBV-positive to EBV-negative patients, or to establish whether different treatment strategies should be used for EBV-positive patients. In this study of 25 selected patients with Richter's transformation, evidence of EBV infection was present in 16% of the patients.

The overall prognosis of patients with Richter's transformation remains poor and the median survival of patients in this study was nine months, which is similar to other reported series [4–6]. One of the aims of this study was to determine whether patients with EBV-associated Richter's transformation had a poorer prognosis than patients in whom the transformation to an LCL could not be shown to be associated with EBV. When we compared the outcome of the four EBV-positive patients with those who were EBV negative, the median survival times were three and nine months, respectively. Although it would

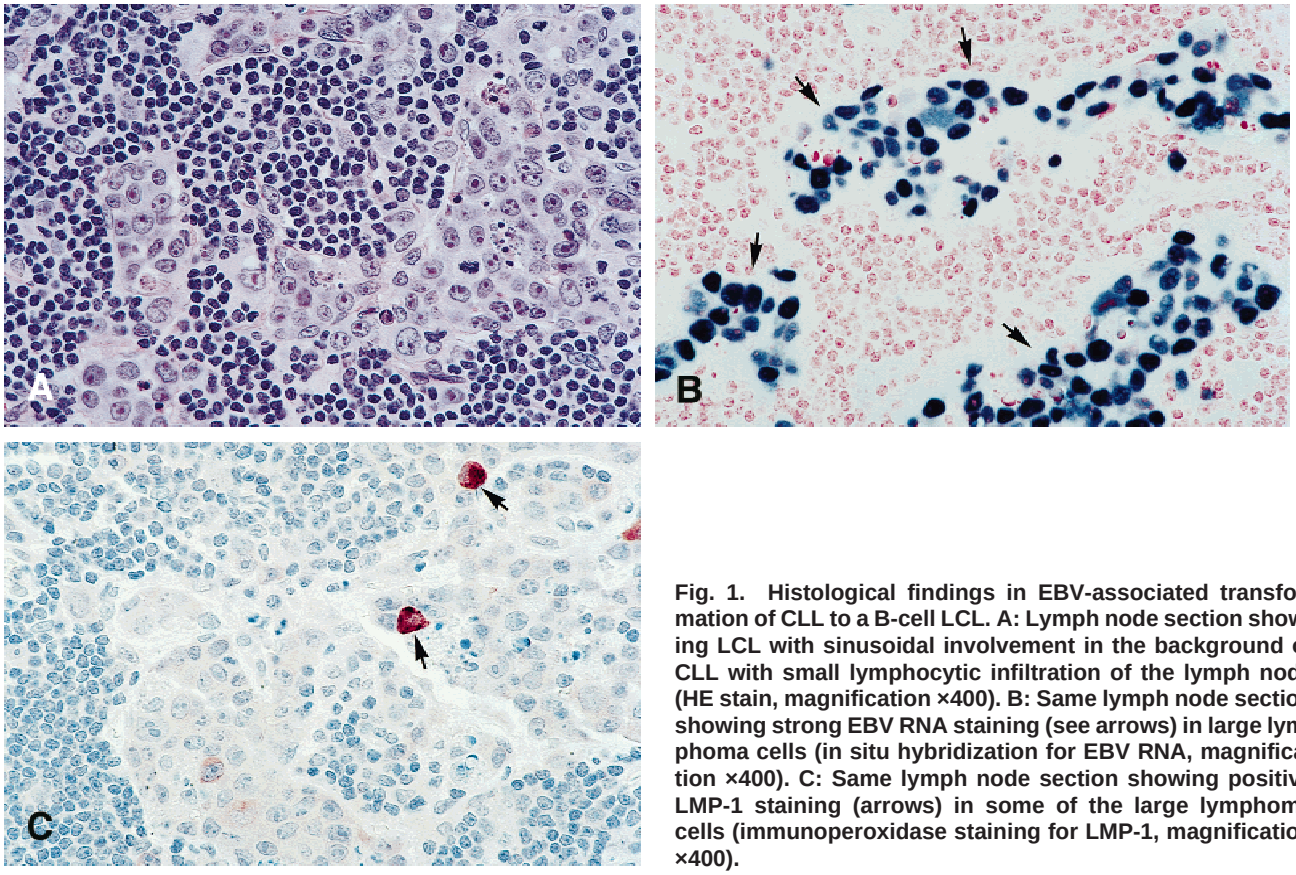

Fig. 1. Histological findings in EBV-associated transformation of CLL to a B-cell LCL. A: Lymph node section showing LCL with sinusoidal involvement in the background of CLL with small lymphocytic infiltration of the lymph node (HE stain, magnification ×400). B: Same lymph node section showing strong EBV RNA staining (see arrows) in large lymphoma cells (in situ hybridization for EBV RNA, magnification ×400). C: Same lymph node section showing positive LMP-1 staining (arrows) in some of the large lymphoma cells (immunoperoxidase staining for LMP-1, magnification ×400).

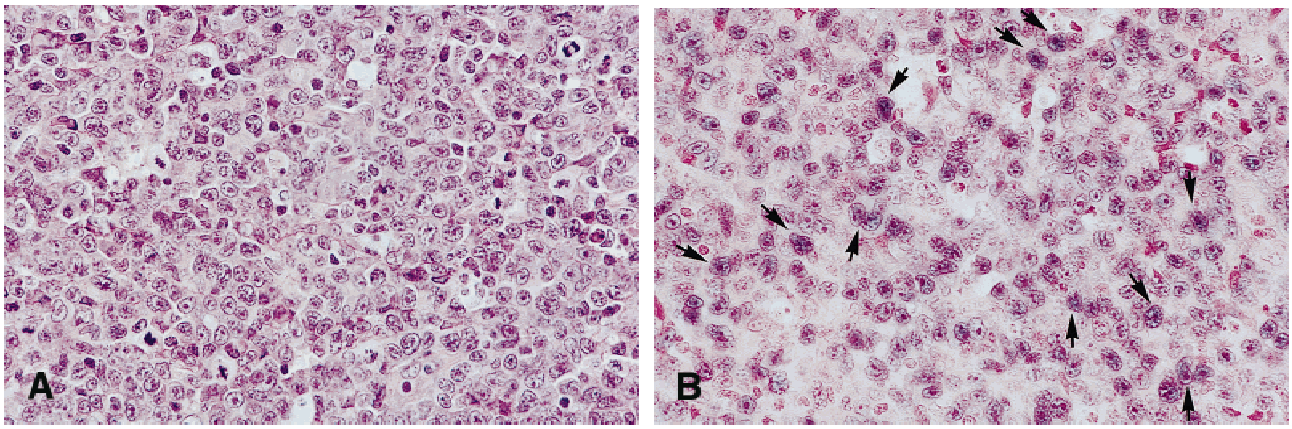

Fig. 2. Histological findings in EBV-associated transformation of CLL to a T-cell LCL. A: Section of a tonsillar mass from a patient with CLL showing features of diffuse LCL (HE stain, magnification ×400). B: Same section showing positive EBV-RNA staining (arrows) in large lymphoma cells (in situ hybridization for EBV-RNA, magnification ×400).

appear that EBV-positive patients might have a poorer survival, this difference was not statistically significant due to the small number of EBV-positive patients.

Rarely, patients with B-cell CLL may develop higher-grade lymphomas of T-cell lineage. Our institution and others have reported a few cases of B-cell CLL or low-grade B-cell lymphomas in patients who subsequently developed higher-grade T-cell neoplasms [24–27]. EBV has been identified in high-grade T-cell lymphomas in other clinical settings [28,29] but has not been shown to be associated with Richter's transformation to a T-cell lymphoma in any of the cases described in the literature. In this series of patients, we found one of the patients to have an EBV-positive LCL of T-cell lineage with a background EBV-negative B-cell CLL. The occurrence of a T-cell lymphoma in this patient may possibly be a sec-

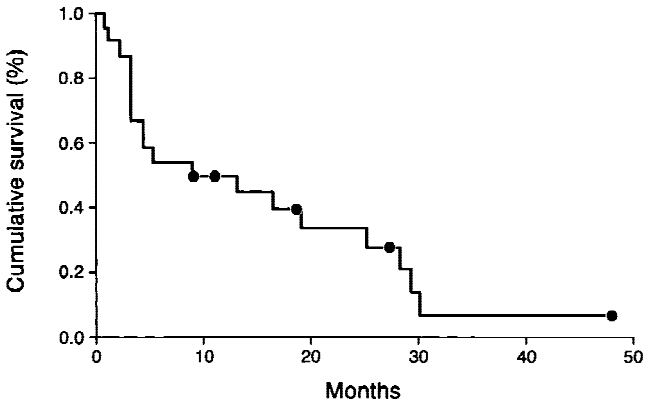

Fig. 3. Overall Survival. Symbols indicate censored values.

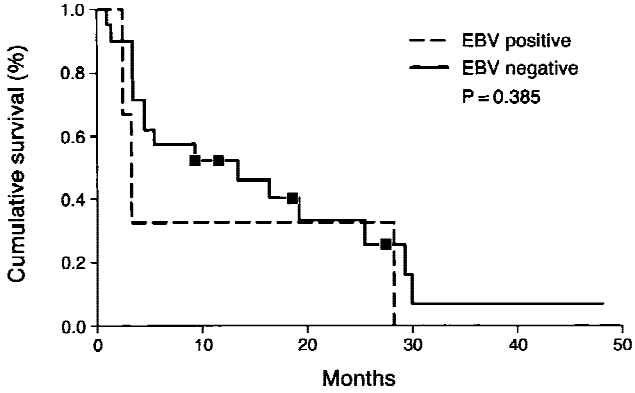

Fig. 4. Survival by the presence or absence of EBV infection. Symbols indicate censored values.

ond, independent event caused by EBV infection of T-cells or a lymphoid stem cell at a stage of maturation before lineage commitment. Alternatively, it may be indirectly related to the CLL due to immune defects caused by the CLL or therapy for the CLL that may have predisposed this patient to the high-grade lymphoma.

In only one patient was the presence of EBV-positive cells in the specimen at the time of the diagnosis of Richter's transformation known to the clinician. This patient was treated with intravenous followed by oral acyclovir as primary treatment and the patient did not receive any other cytotoxic therapy. He did not respond to this therapy and died eight weeks after the diagnosis of Richter's transformation. In this anecdotal case, treatment with acyclovir for EBV-positive Richter's transformation did not seem to be beneficial. However, as an increasing number of new antiviral agents become available, drugs with activity against EBV should be studied in combination with chemotherapy in patients with EBV-associated Richter's transformation.

In summary, EBV-positive Richter's transformation constituted 16% of the patients in this study. Further insight into the natural history, biology, and treatment of this entity is needed and other treatment modalities for patients with EBV-positive Richter's transformation need to be explored.

REFERENCES

1. Richter MN. Generalized reticular cell sarcoma of lymph nodes associated with lymphatic leukemia. Am J Pathol 1928;4:285–292.
2. Armitage JO, Dick FR, Corder MP. Diffuse histiocytic lymphoma complicating chronic lymphocytic lymphoma. Cancer 1978;41:422–427.
3. Foucar K, Rydell RE. Richter's transformation in chronic lymphocytic leukemia. Cancer 1979;46:118–134.
4. Trump DL, Mann R, Phelps R, Roberts H, Conley C. Richter's syndrome: Diffuse histiocytic lymphoma in patients with chronic lymphocytic leukemia . A report of five cases and review of the literature. Am J Med 1980;68:539–548.
5. Harousseau JL, Flandrin G, Tricot G, Brouet J, Seligmann M, Bernard J. Malignant lymphoma supervening in chronic lymphocytic leukemia and related disorders. Cancer 1981;48:1302–1308.
6. Robertson LE, Pugh W, O'Brien S, Kantarjian H, Hirsch-Ginsberg C, Cork A, McLaughlin P, Cabanillas F, Keating MJ. Richter's Syndrome: a report on 39 patients. J Clin Oncol 1993;11:1985–1989.
7. Rubin D, Hudnall SD, Aisenberg A, Jacobson JO, Harris NL. Richter's transformation of chronic lymphocytic leukemia with Hodgkin's-like cells is associated with Epstein-Barr virus infection. Mod Pathol 1994; 7:91–98.
8. Khan G, Norton AJ, Slavin G. Epstein-Barr virus in Reed-Sternberg-like cells in non-Hodgkin's lymphomas. J Pathol 169:9–14, 1993.
9. Shi ZR, Itzkowitz SH, Kim YS. A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcinoma tissues. J Histochem Cytochem 1988;36:317–322.
10. Myers JL, Kurtin PJ, Katzenstein A-LA, Tazeloor HD, Colby TV, Strickler JG, Lloyd RV, Isaacson PG. Lymphomatoid granulomatosis. Evidence of immunophenotypic diversity and relationship to Epstein-Barr virus infection. Am J Surg Pathol 1995;19:1300–1312.
11. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–481.
12. Gehan EA. Generalized Wilcoxon test for comparing arbitrarily singly censored samples. Biometrika 1965;52:203–223.
13. Foon KA, Thiruvengadam R, Saven A, Bernstein ZP, Gale RP. Genetic relatedness of lymphoid malignancies: transformation of chronic lymphocytic leukemia as a model. Ann Intern Med 1993;119:63–73.
14. Traweek ST, Liu J, Johnson RJ, Winberg CD, Rappaport H. High-grade transformation of chronic lymphocytic leukemia and low-grade lymphoma: genotypic confirmation of clonal identity. Am J Clin Pathol 1993;100:519–526.
15. Matolcsy A, Inghirami G, Knowles DM. Molecular genetic demonstration of the diverse evolution of Richter's syndrome (chronic lymphocytic leukemia and subsequent large cell lymphoma). Blood 1994; 83:1363–1372.
16. Caveriviere P, Mallem O, Saati TA, Delsol G. Reed-Sternberg-like cells in Richter's syndrome express granulocytic-associated antigen (leu-M1). Am J Clin Pathol 1986;85:755–756.
17. Choi H, Keller RH. Coexistence of chronic lymphocytic leukemia and Hodgkin's disease. Cancer 1981;48:48–57.
18. Colby TV, Warnke RA, Burke JS, DorfmanRF. Differentiation of chronic lymphocytic leukemia from Hodgkin's disease using immunologic marker studies. Am J Surg Pathol 1981;5:707–710.
19. Dick FR, Maca RD. The lymph node in chronic lymphocytic leukemia. Cancer 1978;41:283–292.
20. Long JC, Aisenburg AC. Richter's syndrome: a terminal complication of chronic lymphocytic leukemia with distinct clinicomorphologic features. Am J Clin Pathol 1975;63:786–795.

21. Suster S, Rywlin AM. A reappraisal of Richter's syndrome: development of two phenotypically distinctive cell lines in a case of chronic lymphocytic leukemia. Cancer 1987;59:1412–1418.
22. Momose H, Jaffe ES, Shin SS, Chen Y, Weiss LM. Chronic lymphocytic leukemia/small lymphocytic lymphoma with Reed-Sternberg-like cells and possible transformation to Hodgkin's disease. Mediation by Epstein-Barr virus. Am J Surg Pathol 1992;19:859–867.
23. Tsang WYW, Ng CS. Epstein-Barr virus and Reed-Sternberg-like cells in chronic lymphocytic leukemia. Am J Surg Pathol 1993;17:853–854.
24. Strickler JG, Amsden TW, Kurtin PJ. Small B-cell lymphoid neoplasms coexisting with T-cell lymphomas. Am J Clin Pathol 1992;98: 424–429.
25. Lee A, Skelly ME, Kingma DW, Medeiros LJ. B-cell chronic lymphocytic leukemia followed by high grade T-cell lymphoma: an unusual variant of Richter's syndrome. Am J Clin Pathol 1995;103:348–352.
26. Deanne M, Amlot P, Pappas H, Norton JD. Independent clonal origin of T- and B-cell clones in a composite lymphoma. Leuk Res 1991;15: 811–817.
27. Jennette JC, Reddick RL, Saunders AW, Wilkman AS. Diffuse T-cell lymphoma preceded by nodular lymphoma. Am J Clin Pathol 1982; 78:242–248.
28. Medeiros LJ, Jaffe ES, Chen Y-Y, Weiss LM. Localization of Epstein-Barr viral genomes in angiocentric immunoproliferative lesions. Am J Surg Pathol 1992;16:439–447.
29. Cheng AL, Su IJ, Chen YC, Uen WC, Wang CH. Characteristic clinicopathologic features of Epstein-Barr virus-associated peripheral T-cell lymphoma. Cancer 1993;72:909–916.